

Original Article

Sexual dimorphism in *Kinosternon scorpioides* (Linnaeus, 1766) from the Brazilian Amazon

Dimorfismo sexual em *Kinosternon scorpioides* (Linnaeus, 1766) da Amazônia brasileira.

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Abstract

The sexual dimorphism of the *Kinosternon scorpioides* was evaluated using two different techniques (linear and geometric morphometry) from images and linear measurements of the carapace and plastron of adults (male and female). Linear morphometry indicated that the height and width of carapace and plastron are statistically different between sexes, with females being wider and taller. In the evaluation of geometric morphometry, ANOVA demonstrated variation in the size of the plastron and the shape of the carapace and plastron, expressing a tendency in shape for each sex. Sexual dimorphism, therefore, is verified for this species, notably by the plastron. This study indicates an additional tool for the phenotypic knowledge of animals, contributing to the study of threatened populations.

Keywords: turtle, morphometry, Amazon, wildlife.

Resumo

O dimorfismo sexual de *Kinosternon scorpioides* foi avaliado utilizando-se duas técnicas diferentes (morfometria linear e geométrica) a partir de imagens e medições lineares da carapaça e plastrão de adultos (machos e fêmeas). A morfometria linear indicou que as médias de altura da carapaça, largura da carapaça e plastrão são estatisticamente diferentes entre os sexos, sendo as fêmeas mais largas e altas. Na avaliação da morfometria geométrica, a ANOVA demonstrou que existe variação de tamanho do plastrão, e na forma da carapaça e do plastrão, expressando uma tendência de forma para cada sexo. O dimorfismo sexual, portanto, é verificado para esta espécie, notadamente pelo plastrão. Este trabalho indica uma ferramenta adicional para o conhecimento fenotípico dos animais, contribuindo no estudo de populações ameaçadas.

Palavras-chave: tartaruga, morfometria, Amazônia, fauna silvestre.

1. Introduction

The use of some aspects of the morphological characterization remains unclear for some animal species, requiring scientific investigations that will prove the applicability of this information for the taxonomic classification. Technological tools have been used to search for these answers, especially the computational analysis of phenotypic traits obtained from animals.

Kinosternon scorpioides (Linnaeus, 1766) is a freshwater chelonian species with ecological, social, and economic

importance to Amazonian riverine populations. The *Kinosternon* genus gathers small to medium-sized turtles, distributed from the south of the United States to the north of Argentina (Kirkpatrick, 1997; Berry and Iverson, 2011; Spinks et al., 2004). One of the forms of identification of chelonians is the analysis of the carapace and plastron; additionally, the measurements of the length and width of these structures are also used for sex determination or verification of population morphometric

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patterns (Cabrera and Colantonio, 1997; Ceballos et al., 2016; Fachín-Terán et al., 2003; Gosnell et al., 2009; Lovich et al., 2010; Vieira and Costa, 2006; Forero-Medina et al., 2007).

Some authors report that *K. scorpioides* has evident sexual dimorphism, based on macroscopic evaluations of adult individuals, evidencing males are generally larger than females, with a longer tail and concave plastron, and females with a smaller tail and straight plastron (Castro, 2006; Pereira and Lemos, 2007; Ferrara et al., 2017). However, these data have been taken through one-dimension measures, disregarding more subtle variations in the body structures.

Geometric morphometry is a technological tool of computer graphics that allows the elucidation of variation in the shape of the body not diagnosed by traditional morphometry, testing and describing the differences in phenotypes (Fornel, 2010; Parés-Casanova, 2014). Despite the successful application of this method to discover sexual dimorphism in many taxa, few applications have been made for turtles (Valenzuela et al., 2004; Ferreira-Júnior et al., 2011).

Considering that information on Brazilian populations of the kinosternidae family is scarce and that anthropic pressures are frequent on the species, morphometric analyses come to collaborate and subsidize management plans, directing captive breeding strategies, contributing to the conservation of Amazonian biodiversity. Thus, this study aimed to compare linear and geometric morphometry for the applicability in the diagnosis of the sexual differentiation of *K. scorpioides*.

2. Materials and Methods

2.1. Sample collection

Kinosternon scorpioides specimens were collected from four populations in the eastern region of the Amazon (Pinheiro, São Bento, and Anajatuba, in Maranhão state; and Belém, in Pará State). The animals from the State of Maranhão were captured manually in the Environmental Preservation Area – APA Baixada Maranhense, and those from Belém were captured in the Rodrigues Alves Forest and the Emílio Goeldi Museum in Pará (see Table 1 and Figure 1). The adult (greater than 10 cm carapace length) specimens were taxonomically identified using the Rueda-

Almonacid et al. (2007) and sexing by visual observation of the tail and plastron.

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2.2. Morphometric analyses

Animals were separated by sex based on traditional linear biometrics (length and width of carapace and plastron; height), using a digital caliper, totaling 177 chelonians. The data were tabulated in an Excel spreadsheet 2010. Assuming that these measurements correctly define the sex, we submitted the data to the Kolmogorov-Smirnov normality test ($D < 0.048647$ Pr $> D > 0.1500$); after normality was verified, the data were submitted to ANOVA and Tukey test by the Statistical Analysis System (SAS).

The body shape variation was analyzed by geometric morphometry, based on landmarks and multivariate statistical methods from photographic images (Sony model H300, optical zoom 35x), maintaining the distance of 30cm between the lens and the animal, as the standard method. To obtain the images, the animals were arranged on a surface with the head turned to the left, positioned dorsally for visualization of the carapace, and ventrally for observation of the plastron. The images were organized and standardized in an image editor, with a resolution of 800x600 pixels. They are transferred to the TpsDig 2.16 program (Rohlf, 2010) with the definition of 24 and 21 fixed landmarks or anatomical landmarks for the carapace and plastron, respectively, according to established descriptions (Claude et al., 2004; Valenzuela et al., 2004; Ferreira-Júnior et al., 2011). Once each anatomical landmark is defined and located, a set of Cartesian coordinates was generated for each point (see Figure 2).

In the MorphoJ 1.06d® program (Klingenberg, 2008), the generated coordinates were overlapped by Generalized Procrustes analysis, producing a matrix with the scores of partial deformations. This procedure removes the variation in size, position, and orientation, transforming it into a unit of centroid size (square root of the sum of squares of the distances of each reference point from the centroid),

Table 1. Sample distribution of *Kinosternon scorpioides*. São Luiz - MA, Brazil, 2019.

Sites		Geometric morphometry						Linear morphometry		
		shell			plastron			M	F	Total
		M	F	Total	M	F	Total			
Maranhão	Pinheiro	11	39	50	10	39	49	11	39	50
	São Bento	18	30	48	19	31	50	16	34	50
	Anajatuba	11	36	47	11	39	49	11	39	50
Pará	Belém	13	14	27	13	13	26	10	17	27
Total		53	119	172	53	122	175	48	129	177

Legend: M = male; F = Females.

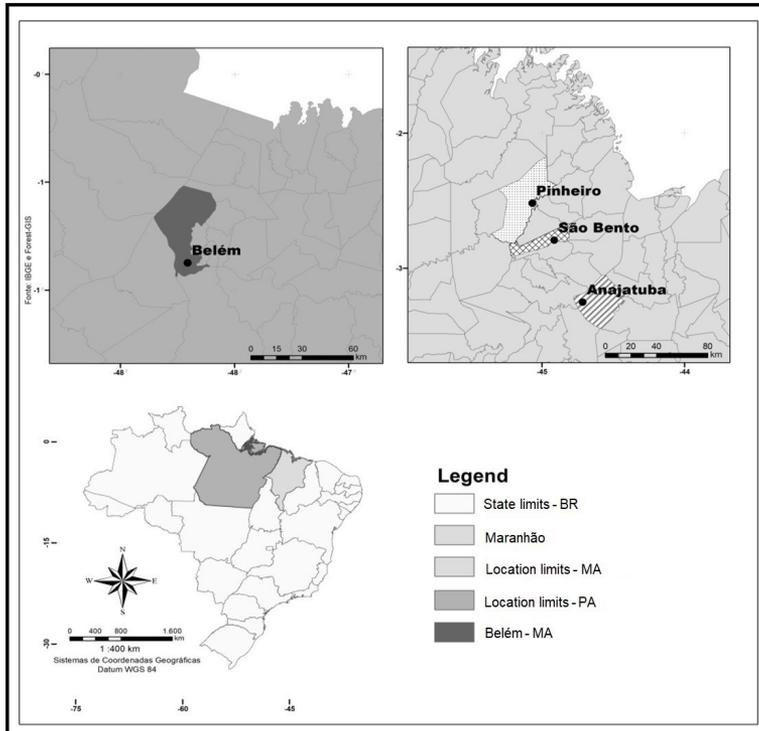


Figure 1. Geographical distribution of *Kinosternon scorpioides* collection points. São Luiz -MA, Brazil, 2019.

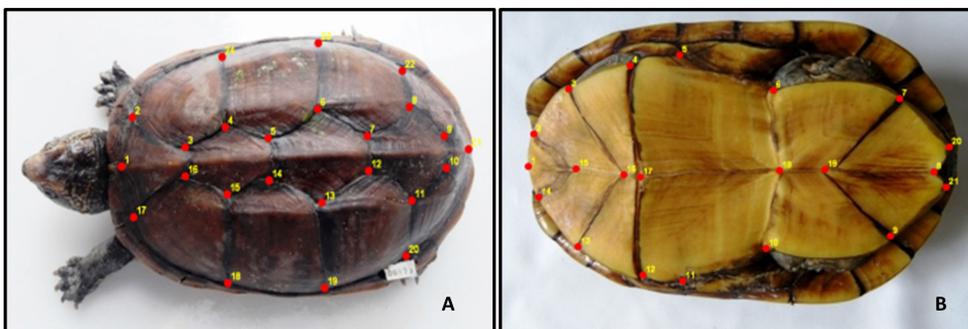


Figure 2. Location of the landmarks arranged in carapace - A and plastron - B of *Kinosternon scorpioides*. São Luiz-MA, Brazil, 2018.

allowing to extract only the shape variation from the data set (Bookstein, 1991).

To verify the sexual dimorphism, principal component analysis (PCA) and discriminant function analysis (DFA) were performed with a permutation test of 1,000 interactions for 172 carapace and 174 plastron samples, regardless of the place of origin. Gender-related size and shape differences were tested using variance analysis (ANOVA) also using Morphoj 1.06d program®.

3. Results

The average carapace length ranged from 13.66 to 13.85 cm for females and males, respectively, with no statistically significant difference in the comparison between

these means, using $p < 0.05$ (see Table 2). The average carapace width showed a significant difference between the sexes, with females being wider than males (see Table 2).

The plastron length of females tends to be larger than that of males, although this is not associated with a statistical difference between the sexes. When analyzing the width of this same structure, the males presented lower measurements than the females, being statistically different from each other. A significant variation was also evidenced for height, with males showing a lower mean than females (see Table 2).

The analysis of variance (ANOVA) on the data of geometric morphometry, related to sex, showed no significant differences concerning the size of the centroid for carapa ($F_{0.43} = 1$; $p = 0.51$), however, for the plastron ($F_{26} = 1$; $p < 0.0001$) there

Table 2. Linear measurements of carapace and plastron of males and females of *Kinosternon scorpioides*. São Luiz-MA, Brazil, 2019.

VALUES	CL (cm)		CW (cm)		PL (cm)		PW (cm)		Height (cm)	
	F	M	F	M	F	M	F	M	F	M
Average	13.66 ^a	13.85 ^a	9.08 ^a	8.80 ^b	12.04 ^a	11.97 ^a	7.43 ^a	6.69 ^b	4.95 ^a	4.56 ^b
Maxim	16.70	16.90	11.00	13.50	15.30	14.40	13.30	9.47	6.30	5.90
Minimum	10.10	10.17	6.70	7.70	5.30	10.33	4.60	5.34	3.08	3.56
St. dev.	1.23		0.85		1.87		1.48		0.57	
p*	0.26		0.027		0.82		0.0023		<0.0001	

Legend: CL = carapace length; CW = carapace width; PL = plastron length; PW = plastron width; M = male; F = female; *p<0.05. Different letters- statistical difference between means. Equal letters mean without statistical variation and different letters with statistical variation.

was statistical variation. There was difference between males and females for the shapes of carapace ($F_{4,45} = 44, p < 0.0001$) and plastron ($F_{17,06} = 38; p < 0.0001$).

The first two components of the PCA applied to the morphometric data from the carapace corresponded together to 46.93% of the total variation, and 30.65% of the variation when applied to the morphometric data from the plastron (see Figures 3I - A and B). In the evaluation of the carapace, PC-1 (28.75%) presents individuals who have a shortening of the cranial shields and enlargement of the caudal part, and PC-2 (18.65%) showed enlargement of the cranial plates and posterior region close to the mean (Figure 3I - C). For the plastron, PC-1 (17.86%) represented individuals with cranial shortening, lateral enlargement (abdominal plates), and the caudal region close to the average, and PC-2 (12.79%) reflected a wider cranial structure, with lateral narrowing and caudal extension, with wider and longer cranial plaques than the average observed (see Figure 3I - D). Males and females are distributed along the two axes (positive and negative), overlapping these individuals in the principal component graph, especially for dorsal structure.

Regarding the Discriminant Function Assessment (DFA) of males and females, the values for carapace and plastron were respectively: distance of Procrustes: 0.02026167/0.02832625; Mahalanobis: 2.2570/3.0984; and $p < 0.0001/p < 0.0001$ indicating differences in shape between males and females for both structures evaluated.

The cross-validation classification table of DFA was 77.04% in females and 72% in males for carapace, and 90.16% in females and 83.02% in males for plastron (see Table 3). The histogram of frequencies through the "cross-validation" of DFA showed overlap between males and females (see Figure 3II - A and B), similar to that observed in PCA. In the evaluation of the wireframe chart of shape for DFA, there was a slight tendency laterally in the shape of the carapace of males concerning females (see Figure 3II C) and with the male plastron presenting narrowing between the abdominal and femoral plates, as well as an increase in the size of the anal scute (see Figure 3II D).

4. Discussion

Although the linear morphometry data of *K. scorpioides* reveal that males have higher carapace length averages,

following what is proposed in the literature for the species (Barreto et al., 2009; Ferrara et al., 2017), no statistically significant variation was evidenced for this parameter concerning gender. In general, terrestrial and semiaquatic turtle males are larger than females, while in aquatic species, males are smaller (Berry and Shine, 1980). For example, males (13.8cm) from *Kinosternon leucostomum postinguinale* have average carapace lengths longer than females (11.8cm) (Ceballos et al., 2016), while in the *Podocnemis unifilis*, females ($x = 35$ cm) have longer shells than males ($x = 26.4$ cm) (Fachín-Terán and Vogt, 2004).

Kinosternon scorpioides is part of the group of small turtles of the New World, with the average size of males and females being inferior to other freshwater turtles, and according to Rueda-Almonacid et al. (2007), they do not exceed 15cm in length. Forero-Medina et al. (2007). Studied the same species on the Island of San Andrés/Colombia (*Kinosternon scorpioides albugulare*) and showed that most individuals had between 11 and 13 cm of carapace length, with males presenting higher lengths than females.

The plastron length did not present differences in sex, although the lowest mean length of this structure occurred in males (see Table 2). This situation was also verified for *K. scorpioides scorpioides* from Ilha de Marajó- Brazil (Silva da Silva et al., 2021), in which females had a longer plastron than males.

This variation is possibly related to reproductive issues since they would need a shorter plastron to efficiently expose their tail and sexual organs, ensuring the success of copulation; this is a reproductive strategy (Silva da Silva et al., 2021). In the population study of *P. unifilis*, the average length of males plastron was also lower than that of females (Fachín-Terán and Vogt, 2004). However, for the species *K. s. albugulare*, there were no differences between genders for the same region (Forero-Medina et al., 2007).

Regarding the measurements of the width of the carapace and plastron and the height of the carapace, the results showed that males and females are different from each other in the comparison of these morphological variables. In these measurements, the females were wider and taller than the males. This morphological difference was also observed in females of *Kinosternon s. scorpioides* (Silva da Silva et al., 2021), *Trachemys scripta elegans* (Viera & Costa, 2006), and *Podocnemis unifilis* (Fachín-Terán & Vogt, 2004). On the other hand, males of *Kinosternon leucostomum*

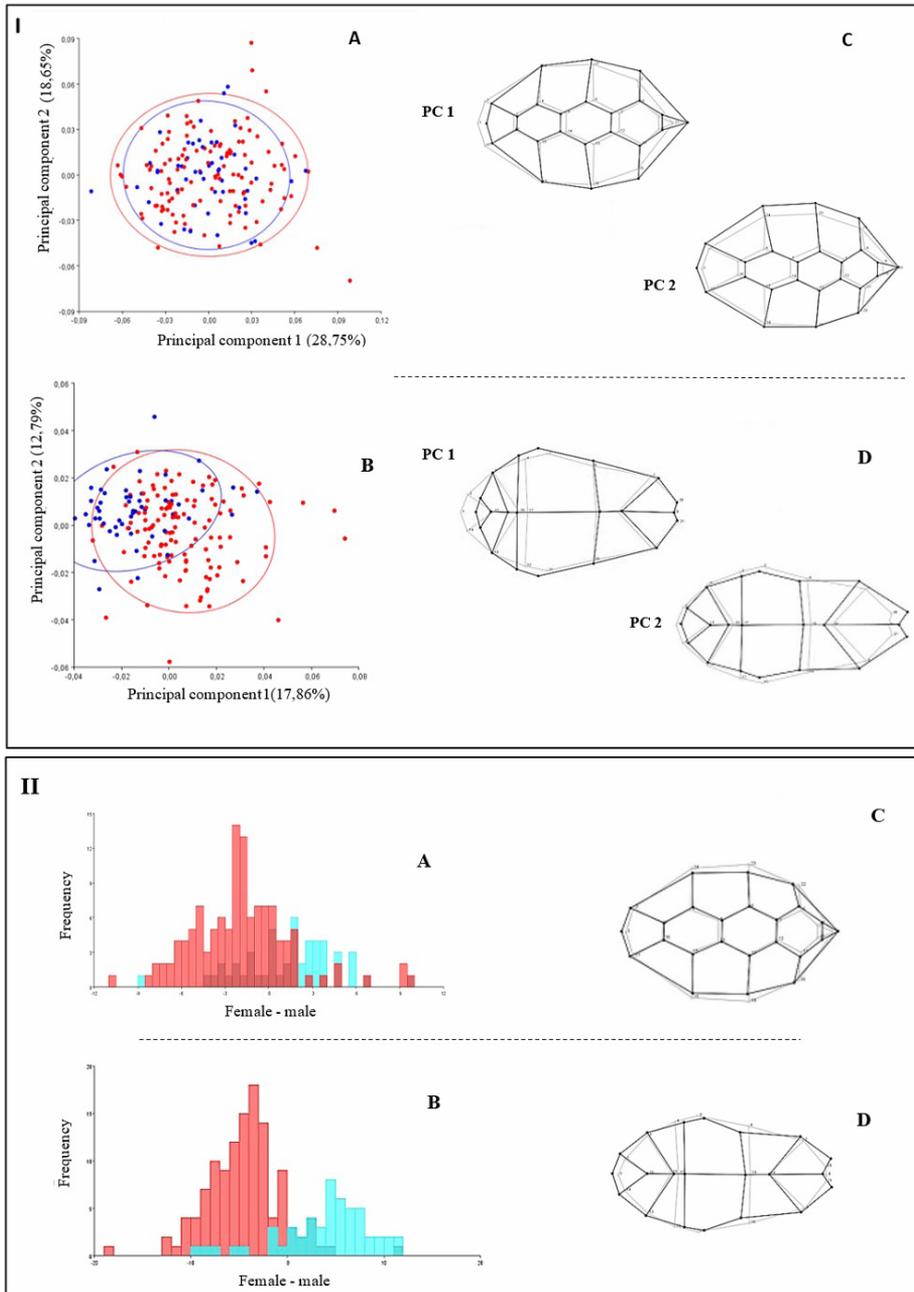


Figure 3. I- Principal Component Analysis/wireframe chart of shape (carapace - A and C; and plastron B and D) and II- Discriminant/Histogram/wireframe function analysis of shape (carapace - A and C; and plastron B and D) of *Kinosternon scorpioides*. São Luiz-MA, Brazil, 2019. Legend: medium-gray line, black-variation line, blue-male, red-female.

Table 3. Classification of the Discriminant function analysis “cross-validation” between sexes, carapace and plastron of *Kinosternon scorpioides*. São Luiz, 2019.

Sex a priori	Cross-validation					Total
	DFA Result - Carapace		Total	DFA Result - Plastron		
	Female	Male		Female	Male	
Female	94 (77.04%)	28 (22.95%)	122 (100%)	110 (90.16%)	12 (09.84%)	122 (100%)
Male	14 (28.00%)	36 (72.00%)	50 (100%)	09 (16.98%)	44 (83.02%)	53 (100%)

from Colombia (Ceballos et al., 2016) are wider than females, although without statistical variation.

We believe that this variation is related to the need for a larger space to house ovarian follicles and eggs along the oviducts in the reproductive season. This inference is based on Darwin's theory (1871) when he related the evolution of the traits of the sexual dimorphism of species, stating that the selection of fecundity of larger females is due to greater reproductive potential.

About height, males from the species *Podocnemis sextuberculata* (Fachín-Terán et al., 2003), *Podocnemis unifilis* (Fachín-Terán & Vogt, 2004), and *K. scorpioides* (Silva da Silva et al., 2021) are also lower than females. It was previously described (Molina, 1992, Castro, 2006; Pereira and Lemos, 2007) as a trait of sexual differentiation of the chelonians in which males are lower than females (Ferrara et al., 2017). Thus, the trait dimensions: width of carapace and plastron and carapace height can be considered criteria of sexual differentiation for *Kinosternon scorpioides*.

Morphometric analyses showed that the centroid size for the carapace and plastron showed divergent results, with statistical variation only for the plastron when submitted to the analysis variance. However, the same test showed morphological differences in gender in evaluating the shape of the two structures that make up the exoskeleton, carapace, and plastron. Given the above on the linear analysis of individual data, we believe that the statistical variation presented for the width and height of the specimens may have contributed to the presentation of variation in the shape of those structures.

Although the statistical analysis used by the ANOVA test showed differences in shape concerning gender, the principal component analysis was not sensitive to adequately separate males from females, with an overlap of some individuals, leading us to realize that the typical format by sex should not be inferred for any of the views (dorsal or ventral) through this analysis. However, it is essential to highlight that the plastron presented overlap smaller than the carapace, where females are more willing to the positive side of the X-axis, demonstrating that they are mostly wider laterally than males, which were concentrated on the negative side of the graph. These indicators corroborate the data obtained from the linear analysis, with males presenting plastron narrowing, a morphological variation also observed macroscopically compared with the female plastron. Macale et al. (2011) analyzed the morphology of the Egyptian Tortoise (*Testudo kleinmanni*). They evidenced the similarity with the results observed in our study, morphological variation linked to sex: in males, the plastron is narrowed, and the carapace assumes a hemispheric shape, while in females, the plastron is wider, and the carapace has a caudal narrowing.

The overlap of groups was also observed in the DFA, in which it was not possible to separate individuals in their entirety from gender. The Distance of Procrustes between the two groups was close to zero, indicating weak variation in shape. However, cross-validation data showed a good percentage of distinction for carapace and excellent sexual differentiation for the plastron. And similar to that observed in PCA, the DFA, the best results were obtained using the ventral region of the chelonian. According to

Myers et al. (2006), the plastron is a structure that has an ideal pattern for morphometric analysis due to its flat format, ensuring suitable reference locations for analysis. The best cross-validation and a lower overlap of groups in PCA and DFA occurred when the evaluation of this structure for *K. scorpioides* occurred.

The DFA wireframe graph (see Figure 3II - C and D) shows the morphological tendency for each sex, with a more evident distinction for the ventral region. The carapace of females tends to be wider laterally and shorter cranially than males. And for the plastron, the males of *K. scorpioides* tend to be narrower than females. These morphological aspects are congruent to the findings in linear morphometry.

In addition, a lateral extension of the anal scute is perceived in males, evidencing the formation of a "V" notch, which is not evident in females (see Figures 3I and 3II - D). In a macroscopic analysis performed for the same species, Berry and Iverson (2011) describe this notch as present only in populations from South America and that they are reduced in females, a situation found in our study. This notch highlighted by geometric morphometry may be related to reproductive issues because this format favors copulation, allowing the fit and support of the male to facilitate the passage of the sexual organ to the interior of the female cloaca at the time of copulation. This theory was also mentioned by Macale et al. (2011) when verifying this same characteristic in adult males of the Egyptian tortoise, indicating that this shape would be related to tail movement, facilitating copulation. Thus, this shape of the caudal region of the plastron of *K. scorpioides* males can also be used as a sexual distinguishing feature in individuals from Brazil.

5. Conclusion

The analyses showed that from the geometric morphometry, it is possible to evaluate the variations already described in the literature as linked to sex for the *K. scorpioides* species, being the females wider and taller than the males. Additionally, this methodology improves the observation of the variation since it recorded that the plastron of males has a peculiar and distinctive narrow shape, demonstrating to be the best external to be used in the sexual differentiation of the species.

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